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(54) Title: COSMETIC OR PHARMACEUTICAL COMPOSITION USEFUL IN INHIBITING OR DELAYING HUMAN ALOPECIA BY MEANS OF TOPICAL APPLICATION OF THE COMPOSITION

(57) Abstract: Cosmetic or pharmaceutical composition administered topically on the scalp, useful for the treatment of alopecia containing an enzyme that causes the decomposition by hydrolysis of the dihydrotestosterone thus preventing the latter causing the atrophy and successive death of the hair.



WO 01/66702 A1

Title

"COSMETIC OR PHARMACEUTICAL COMPOSITION USEFUL IN INHIBITING OR DELAYING HUMAN ALOPECIA BY MEANS OF TOPICAL APPLICATION OF THE COMPOSITION"

5 Technical Field

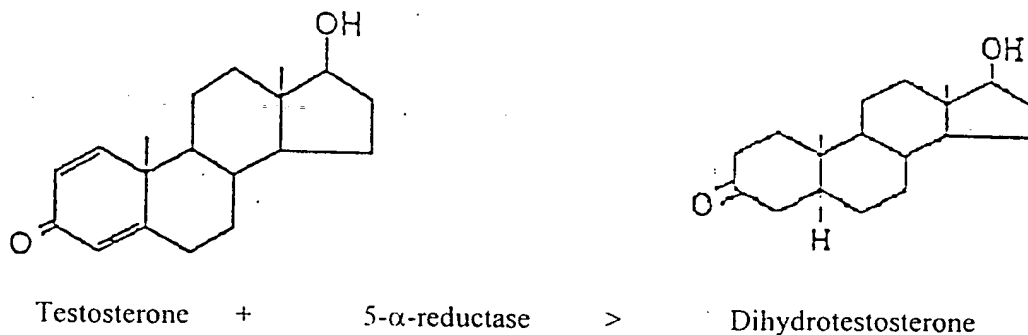
The present invention relates to a product that has applications in cosmetic or pharmaceutical treatment in order to fight alopecia or hair loss.

More particularly, the present invention refers to an enzymatic composition that allows the causes that determine hair loss to be fought with success.

- 10 Hair loss in men is due to various reasons and one of the most accepted attributes hair loss to the action of particular enzymes that prevent the development of piliphric bulbs.

In men over a certain age testosterone is transformed into dihydrotestosterone, due to the action of the enzyme 5- α -reductase,.

15



- 20 The latter moves from the prostate or testicles, where the testosterone and the enzyme 5- α -reductase are normally localized and goes to localize in the micro-capillaries of the piliphric bulb, causing the atrophy and death of the bulb with consequent loss of the hair.

Background art

There are two possible ways to oppose this phenomenon: - inhibit the 5- α -reductase or destroy the dihydrotestosterone as it is formed.

Since the α -reductase is found, as was stated above, localized in the testicles and prostate, it is obvious that a topical treatment in these regions is difficult to carry out.

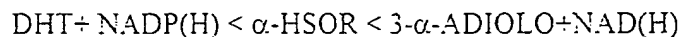
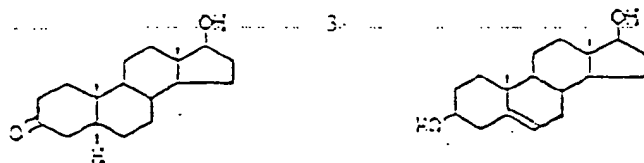
5 It is, therefore, an objective of the present invention to provide a composition which is applied topically on the scalp and prevents localization of the dihydrotestosterone in the piliphtric bulbs.

Disclosure of the invention

The objective of the present invention is achieved by bringing the dihydrotestosterone
10 into contact with an enzyme that causes it to decompose by oxidation, thus preventing the dihydrotestosterone from causing the atrophy and the successive death of the hair.

Enzymes capable of hydrolysing the dihydrotestosterone are those defined as oxidoreductases, eventually assisted by an oxidoreduction coenzyme, e.g., from NADP (H)/NAD(H). One enzyme among those able to decompose
15 dihydrotestosterone by oxidoreduction, has been demonstrated to be particularly active: 3- α -Hydroxysteroid oxidoreductase or 3- α HSOR.

Without constituting a limitation of the ambit of the invention, we maintain that the decomposition of the dihydrotestosterone is a reversible reaction that depends on the ratio of NADP(H)/NAD(H). The greater the concentration of the NADP(H), the more
20 the reaction goes towards the formation of the 3- α -adiolo, degrading the dihydrotestosterone and transforming the NADP(H) nucleotide into NAD(H) nucleotide. Viceversa, when the concentration of the NAD(H) exceeds the concentration of the NADP(H), the same enzyme behaves in the inverse way and catalyzes the dihydrotestosterone-forming reaction according to the outline:



- 5 The 3- α -hydroxysteroid oxidoreductase can be applied in the form of a lotion, shampoo, or in any other form suitable for a topical treatment. It could be mixed with other compounds well known in the cosmetic art which would favor penetration into the lower layers of the skin of the scalp.

Best mode to carry out the invention

- 10 The present invention will be better understood, and the advantages of the same will be better appreciated, from reading of some examples of its embodiments which are provided by way of example but which should not be interpreted as limiting the application of the invention.

DOSAGE Of the DIHYDROTESTOSTERONE

- 15 the dosage of the dihydrotestosterone is an important stage in the verification of the activity of the enzyme. Determining analytically the reduction of the concentration of the dihydrotestosterone in culture medium is sufficient to verify the effectiveness of the same system.

- The methodology developed was taken from the standard methodology for establishing
20 the dosage of steroids.

High concentrations of dihydrotestosterone are present In culture medium. so dosage by HPLC is advantageous. Vice versa, for a similar determination on plasma or, worse still at the level of the piliphric bulb, where the levels of dihydrotestosterone are necessarily very low, such methodology is insufficient.

For our purposes, however, the methodology developed has been shown to be sufficiently precise.

Below are reported the details of the methodology used for the determination by high resolution liquid chromatography. HPLC:

5	Column:	LiChroART 125-4 Purospher RP 18 and, 5µm	
	Mobile phase:	A: Water	
		B: Acetonitrile	
	Gradient:	0 minutes	75%A-25%B
		20 minutes	50%A-50%D
10		30 minutes	50%A -50% B
	Flow-rate:	1 ml/min.	
	Detector:	UV 220nm	
	Temperature:	28 °C	
	Injection:	10µm	
15	Retention time:	15 mm.	

VERIFICATION Of the DEGRADATION Of DHT by 3-a-HSOR -

hydroxytestosterone dehydrogenase catalyzes the interconversion reaction of the carboxylic and hydroxylic group of the hydrotestosterone. It is a typical oxidative reaction and by doing so reduces NAD. It is therefore possible to measure the oxidation of dehydrotestosterone in the presence of NAD, estimating the reaction kinetics by measuring an increment of absorbance at 340 nm due to the reduction of the same NAD. Moreover it is possible quantitatively to verify the presence of Dihydroxytestosterone in the enzyme solution, before and after the same reaction.

Reagents:

25 - 0.03 M tris-HCl buffered to pH7.2 with 0.001 M EDTA

- 5
- 0.166 M sodium pyrophosphate buffered to pH 9
 - 0.0043 of NAD in purified water for HPLC. The NAD can vary in salt form depending on the degree of oxidation.
 - 0.015% Dihydrotestosterone, preparing 15 mg of dihydrotestosterone in 100 g of absolute alcohol for HPLC.

Enzyme:

Dissolve the enzyme to a concentration of 1mg/ml in 0.03 M tris.HCl pH7,2 with 0.001 M EDTA, the successive dilutions can be carried out with the same buffer solution.

10 Procedure:

Calibrate the spectrophotometer to 340 nm and 25°C -

Each quartz cuvette contains the following amounts:

- | | |
|----------------------------------|--------|
| - 0.166 M of sodio pyrophosphate | 0.6 ml |
| - 0.0043 M NAD | 0.2 ml |
| 15 - Water purified for HPLC | 2 ml - |
| - Enzyme | 0,1ml |

One leaves all to incubate in the spectrophotometer until a temperature equilibrium is reached so as to be able to establish the value of absorbance of the blank.

- At time 0, i.e. at the beginning, add 0,1 ml of the dihydrotestosterone solution, and
- 20 after a few seconds collect an aliquot of 20 µl to inject into the HPLC previously prepared for the dosing of the dihydrotestosterone.

The reaction is followed for 5 minutes and the variations of absorbance of the solution at 340 nm are noted. After five minutes, take another aliquot of 20 µl of solution and inject it into the HPLC again in order to verify the residual amount of

25 dihydrotestosterone remaining after the enzyme reaction.

Calculation of the amount of enzyme:

The amount of enzyme expressed in unit/mg of substance is estimated as follows:

$$5 \quad U/mg = \frac{\Delta A_{340}/min}{6.22 \times mg \text{ Enzyme} / ml}$$

where μA_{340} = variation of the absorbance to 340 nm.

min = minute

10 mg = milligrammi

ml = mls of the reaction solution

6.22 = conversion factor

Results:

The results of the dosage of the dihydrotestosterone are shown in table 1

15 TABLE 1

The first column shows values relating to the absorbance at 340 nm of solution during the enzymatic kinetics phase, while the second column reports time expressed in minutes and seconds.

a

20	1.2	0
	1.34	30
	1.5	60
	1.65	90
	1.81	120
25	1.94	150
	2.1	180
	2.24	210

2.4 240

2.55 270

2.66 300

TABLE 2

5 Results relating to the dosage of DHT the before and after enzyme kinetics with HSOR

Theoretical Concentration of DHT in medium: 0.015 mg/ml

Concentration shown instrumentally

At time 0: 0.012 mg/ml

10 At time 100": 0.009 mg/ml

At time 200": 0.007 mg/ml

At time 300": not determinable

EVALUATION OF THE ANTI-DHA ACTIVITY AND THE INCREASE AND DEVELOPMENT OF HAIR IN SUBJECTS AFFECTED BY ALOPECIA

15 ANDROGENETICA

Materials and methods

The study was carried out on thirty males aged between 25 and 40 years clearly affected by alopecia androgenetica and at various stages of alopecia.

The group was divided into two homogenous subgroups, each of 15 people.

20 Treatment

Two series of samples in phials were prepared containing 10ml of the following solutions in distilled water:

Solution A

Hydrolyzed Chitin 2%

25 Tween 20.8%

Capsico resin oil 0.1%

Preserving system pH 7.5

Solution B

Solution A with addition of 2.5 mg/10ml hydroxysteroid dehydrogenase before the
5 phial was used.

In order to make the phials with enzyme unrecognizable to the patients, 25 mg' of powder was added to the phials. The A phials contained only sodium bicarbonate, the B sodium bicarbonate added to the enzyme.

The treatment continued for a period of six months, during which the patients used
10 one 10 ml phial in the morning and one in the evening.

Assessment

The assessment was carried out by selecting a surface of 25cm^2 of the scalp for every subject on which the following measurements were carried out at intervals of 15 days: hairs counted and length measured. In this area the hairs were cut to the same length
15 before the test began.

Results

The treatment carried out with the of the type B phials, in which the enzyme hydrosteroid dehydrogenase (HSOR) was present demonstrated good effectiveness in regrowing hair; in fact, the average regrowth length after six months of treatment was
20 7.5mm.

Moreover beyond the greater average regrowth, there was also an increase in the average number of hairs, from the 100 initially to 185 finally in the group treated with with phial B, while those treated with the solution A went from the 100 initially to the 75 finally.

CLAIMS

1. Cosmetic or pharmaceutical composition administered topically on the scalp, useful for the treatment of alopecia characterized by containing an enzyme that causes the decomposition by hydrolysis of the dihydrotestosterone thus preventing the latter
5 causing the atrophy and successive death of the hair.
2. Cosmetic or pharmaceutical composition administered topically on the scalp, useful for the treatment of alopecia, according to Claim 1, characterized by the enzyme being an oxidoreductase.
3. Cosmetic or pharmaceutical composition administered topically on the scalp, useful
10 for the treatment of alopecia, according to Claim 1 or 2, characterized by the enzyme being 3- α -hydroxysteroid oxidoreductase
4. Cosmetic or pharmaceutical composition administered topically on the scalp, useful for the treatment of alopecia, according to Claims from 1 to 3, characterized by being used together with an oxidoreductase coenzyme.
- 15 5. Cosmetic or pharmaceutical composition administered topically on the scalp, useful for the treatment of alopecia, according to Claim 1, characterized by the coenzyme being the NADP(H)/NAD(H) system.
6. Cosmetic or pharmaceutical composition administered topically on the scalp, useful for the treatment of alopecia according to one or more of the previous Claims,
20 characterized by comprising surfactants, cheratin and preservative.
7. The use of 3- α -hydroxysteroid oxidoreductase for the preparation of compositions administered topically for the treatment of alopecia
8. The use of 3- α -hydroxysteroid oxidoreductase in a mixture with the coenzyme NADP(H)/NAD(H) for the preparation of cosmetic or pharmaceutical compositions

administered topically for the treatment of alopecia due to the localization of dihydrotestosterone in the capillaries of the pilipheric bulbs.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IT 01/00106

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/02 A61K7/06 A61K38/43

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE WPI Section Ch, Week 198709 Derwent Publications Ltd., London, GB; Class B04, AN 1987-059542 XP002169772 & JP 62 012721 A (MORITA M), 21 January 1987 (1987-01-21) abstract</p> <p>---</p>	
A	<p>US 5 756 092 A (BERNARD BRUNO ET AL) 26 May 1998 (1998-05-26)</p> <p>-----</p>	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 62012721 A	21-01-1987	NONE	
US 5756092 A	26-05-1998	FR 2732597 A CA 2173544 A EP 0736300 A	11-10-1996 06-10-1996 09-10-1996